Microbial Synthesis of Silver Nanoparticles and its Antimicrobial activity.

Vineetha K[1], Bharath Raj K C[2], Harish K H[3], Prashant Nayak[4*]

[1]Department of Pharmaceutics, Srinivas College of Pharmacy, Valachil, Mangaluru, Karnataka, India.

[2]Nitte (Deemed to be University), NGSM Institute of Pharmaceutical Sciences (NGSMIPS), Department of Pharmacy Practice, Mangaluru, India.

[4]Department of Pharmaceutics, KLE College of Pharmacy, Hubli (A Constituent unit of KLE Academy of Higher Education and Research, Belgavi), Karnataka, India.

[3]Nitte (Deemed to be University), NGSM Institute of Pharmaceutical Sciences (NGSMIPS), Department of Pharmaceutics, Mangaluru, India.

Abstract:

The present research work aims at development of reliable and eco friendly method for synthesis of silver nanoparticles. Recently, microorganisms have been explored as potential biofactory for Bio synthesis of silver nanoparticles. In this study, we used soil samples of marine samples and paddy field as source of bacteria, our research showed that extracellular biosynthesis of metallic silver nanoparticles by the reduction of aqueous Ag^+ using soil and sea samples as potential candidates for the rapid synthesis of silver nanoparticles. Silver nanoparticles biosynthesis by reduction of silver ion coming in contact with the cell filtrate was fast as it formed within few minutes. UV-visible spectrum of the aqueous media obtained from the above bacteria containing silver ion showed a peak around 420 nm corresponding to the absorbance of silver nanoparticles. Scanning electron microscopy (SEM) micrographs showed formation of well-dispersed silver nanoparticles in the range of 20–70 nm. Antibacterial activity of silver nanoparticles was comparable to amoxicillin.

Keywords: Silver nanoparticle, nanotechnology research, biological synthesis, Scanning electron microscopy, Antibacterial activity.

Introduction:

Nanotechnology is an emerging field in the area of interdisciplinary research, especially in biotechnology. The synthesis of silver nanomaterials/nanoparticles extensively studied by using chemical and physical methods, but the development of reliable technology to produce nanoparticles is an important aspect of nanotechnology. Biological synthesis process provides a wide range of environmentally acceptable methodology, low-cost production and minimum time required. At the same time the biologically synthesized silver nanoparticles has many applications includes catalysts in chemical reactions [1], biolabeling, antimicrobial agent, electrical batteries [2] and optical receptors [3,4]. Microbial source to produce the silver nanoparticles shows the great interest towards the precipitation of nanoparticles due to its metabolic activity. Of course, the precipitation of nanoparticles in external environment of a cell, it shows the extracellular activity of organism. Extracellular synthesis of nanoparticles using cell filtrate could be beneficial over intracellular synthesis, the fungi being extremely good candidates for extracellular process and also environmentally friendly. There are few reports published in literature on the biosynthesis of silver nanoparticles using fungal as source. The use of bacterial strain in the bio-manufacturing process has the advantage that is ease of handling than the fungus [6,7,8]. In this present work, microbial production of silver nanoparticles was investigated using the unknown bacterial strain from marine (sea) and paddy field soil. This research work implies the different medium composition for production of silver nanoparticles and characterization of particles by UV- visible spectrometer. To our knowledge, extracellular synthesis of silver particles by unknown bacterial strain with two different basal medium compositions has not been reported so far.

When microorganisms exposed to high metal ion concentration, they develop strategy to detoxify them by reducing in to zero valent atoms. This unique property of microorganism is used to synthesize metal nanoparticles. Various microbes such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and thermophilic fungus *Humicola sp* are reported for the synthesis of metal nanoparticles with uniform size and better stability. These economic and ecofriendly marine and paddy field microorganisms are sources of potential micro flora which have great potential in synthesizing metal nanoparticles.[10]

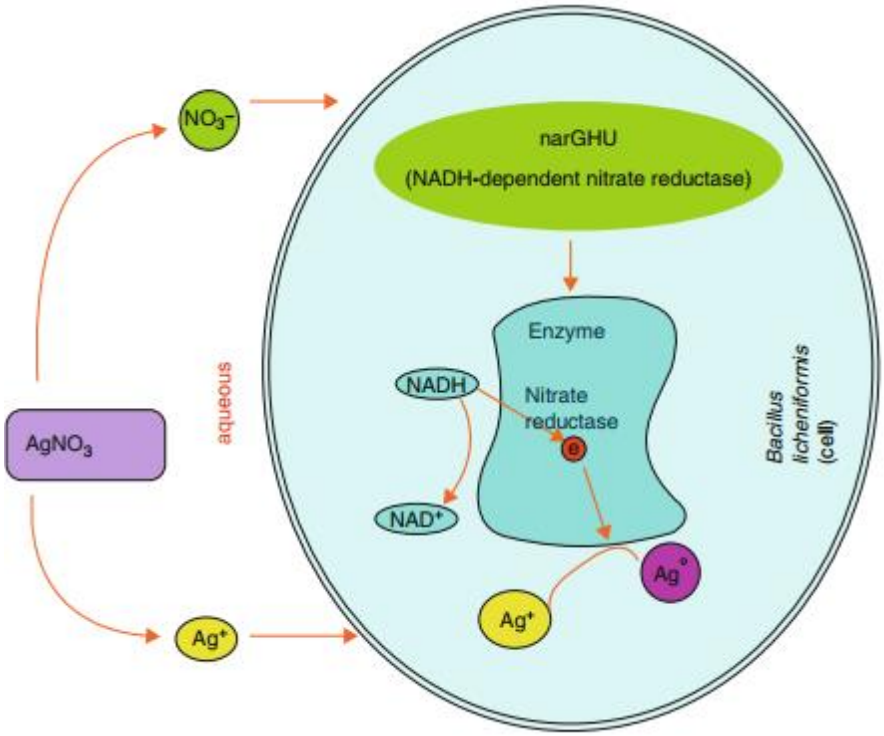

Figure 1: A hypothetic diagram of how silver ions are reduced to silver atom by the enzyme

Materials and methods:

Source of microorganism

The bacterial strain was obtained from the soil from paddy field from Puttur and marine source from Ullal beach, Mangalore India. The samples were maintained in nutrient agar medium (HiMedia, Mumbai, India) and nutrient agar slant at 27°C as well as subcultured from time to time to regulate its viability in the microbiology laboratory during the study period. The samples were named as soil samples and marine samples

Isolation and characterization of the strain

The bacterial strain obtained from soil and marine (sea) was isolated using pour plate method in a nutrient agar petri plate. Similar colonies were isolated and put in nutrient broth for further characterization. Further characterization was done by gram's staining using crystal violet, safranin and iodine reagents for both sea and paddy field soil the samples were labeled as paddy field soil and marine sand.

Production of biomass

The bacterial strain from soil and marine samples separately were cultured, to produce the biomass for biosynthesis. Nutrient broth medium was used as a medium for growing the bacterial samples.

Microorganism was cultured in a 250 ml culture flask The culture flasks were incubated on an orbital shaker at 27°C and agitated at 220 rpm. The biomass was harvested after a week clear growth was observed. The culture was collected and centrifuged at 12000 rpm for 10 minutes. The supernatant was subjected to filtration by using filtration assembly of Millipore using 0.22-micron size filter paper; the filtrate was collected and used for the bio-synthesis of silver nanoparticles.[11]

Synthesis of silver nanoparticles

The filtrate of bacterial strain from nutrient broth medium was used for synthesis. The filtrate was added separately to the reaction vessel containing silver nitrate (AgNO3) at a concentration of (1% v/v) 1mM and control (without the silver nitrate, only filtrate) was also run along with the experimental condition. Both the filtrate and silver nitrate solution were used in equal proportion. The reaction was carried out in beaker with magnetic stirrer, the reaction between this filtrate and Ag+ ions was carried out in bright conditions for hours till absorbance of around 420 nm was observed in UV-Visible spectrophotmeter.[12]

Characterization of Silver Nanoparticles

The bio reduction of the Ag+ ions in the solution was monitored and sample of 2ml was withdrawn at different time intervals and the absorbance was measured at a resolution of 420 nm using UV–visible spectrophotometer (UV–1700 series, SHIMADZU) with samples in quartz cuvette.[13]

Particle size measurement

Silver nano particles was measured by scanning electron microscope (S.E.M) Shape and surface morphology of the silver nanoparticles were studied using SEM (JEOL, JSM 50A, Tokyo, Japan). An appropriate amount of silver nanoparticles was mounted onto metal (aluminium) using double-sided adhesive tape and fractured with a razor blade. The samples were sputter-coated with gold/palladium for 120 sec at 14 mA under argon atmosphere for secondary electron emissive SEM and observed for morphology, at an acceleration voltage of 15 KV. [14]

Antimicrobial activity of silver nanoparticles

The antimicrobial activity of silver nanoparticles will be checked by employing agar diffusion method (cup plate method). Culture strains of *Bacillus subtilis* will be grown in the media as the organism will be health it will be used as inoculum. Meantime agar plate is allowed to settle and 6 holes of equal distance is made on agar plate with a sterile cork borer The silver nanoparticles and amoxicillin (reference standard) were incorporated in the agar plates then kept in refrigerator for an hour for diffusion of the samples then it is incubated at 37^0C for 72 hours. The reading was

done in triplicate and zone of inhibition was calculated. [15]

Results and discussions

Isolation and characterization of the strain

Isolation of microorganism was done by pour plate method and kept for overnight incubation and colonies grown were observed under colony counter and check for uniform size colonies these colonies were grown in nutrient broth.

The samples were further differentiated by Gram's staining reaction as shown in table 3

Table 1: Results of Gram's staining

Paddy field soil sample	Gram negative microorganism
Sea sample	Gram positive microorganism

Synthesis of silver nanoparticles

Synthesized silver nanoparticles were subjected at various time intervals and absorbance was recorded since silver nanoparticles have maximum absorption 0f 0.548 which was observed at around 400-420 nanometer as shown in table 4 and figure 2.

Table 2: Absorbance of silver nanoparticles

Wavelength in nm	Observed Absorbance ± SD
0	0.086 ± 0.0009
100	0.234 ± 0.0066
200	0.374 ± 0.0054
300	0.462 ± 0.0090
400	0.548 ± 0.0065
500	0.421 ± 0.0070
600	0.210 ± 0.0070

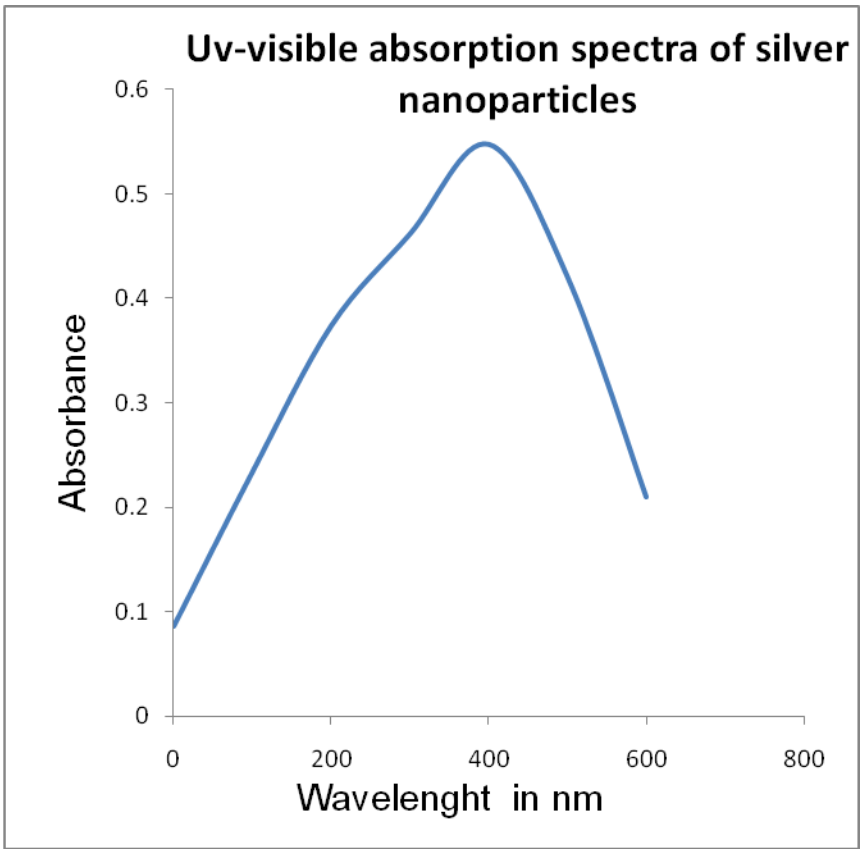

Figure 2: UV-Visible absorption spectra of silver nanoparticles.

Characterization of Silver Nanoparticles

Silver nanoparticles was characterized using SEM (scanning electron microscope)

Figure 3 showed that nanoparticles in the range of 20 nm to 70 nm.

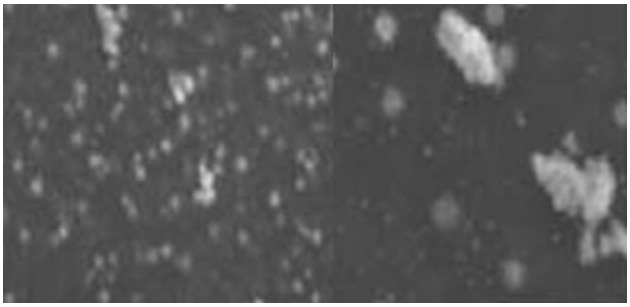

Figure 3: SEM (scanning electron microscope) of Silver nanoparticles.

Antimicrobial activity of silver nanoparticles

Silver nanoparticle showed a zone of inhibition by cup plate method

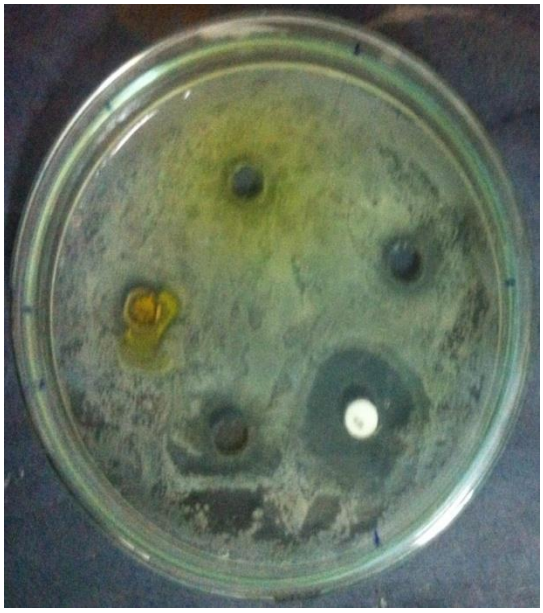

Figure 4: Zone of inhibition of silver nanoparticles by cup plate method

Table 3: Zone of inhibition of silver nanoparticles

Sample	Zone of inhibition *bacillus subtilis* in mm
Silver nanoparticles	18.23 ± .0145
Amoxycillin	23.24 ± .084

As seen zone of inhibition was calculated and silver nanoparticles showed nearly same zone of inhibition as compared to standard amoxicillin. So silver nano particles have some antibacterial activity.

Conclusion

Synthesis of silver nanoparticles from extracellular source of microorganism showed the synthesized silver nanoparticles in the range of 20-70 nm. This method was ecofriendly and economical. Synthesized nanoparticles showed good zone of inhibition was comparable to amoxicillin. Further studies on toxicity and *in-vivo* studies will have to be conducted for assessing the safety and efficacy of synthesized silver nanoparticles.

Conflict of Interest:

The authors declare no conflict of interest.

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Authors:

First Author- Vineetha K, Department of Pharmaceutics, Srinivas College of Pharmacy, Valachil, Mangaluru, Karnataka, India.

Second Author- Bharath Raj K C, Nitte (Deemed to be University), NGSM Institute of Pharmaceutical Sciences (NGSMIPS), Department of Pharmacy Practice, Mangaluru, India

Third Author- Harish K H, Department of Pharmaceutics, KLE College of Pharmacy, Hubli (A Constituent unit of KLE Academy of Higher Education and Research, Belgavi), Karnataka, India.

Corresponding author- Prashant Nayak, Nitte (Deemed to be University), NGSM Institute of Pharmaceutical Sciences (NGSMIPS), Department of Pharmaceutics, Mangaluru, India.